The Effects of Profilin-1 Depletion in Various Signaling Pathways

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INTRODUCTION
Cancer is a very prevalent and dangerous disease, with many sufferers and an estimated 1,685,210 new cases in the United States each year [1]. In the case of breast cancer specifically, 1 in 8 women are expected to be victims of the disease. Cancer results in costs of $125 billion annually and is expected to grow to as much as $156 billion by 2020. Only with more efficient treatment options can we hope to increase survival rate and keep costs down.

Current research has pointed to gene activations with respect to cancer growth and metastasis. This new area has been invested in greatly, however with the nature of cancer (in both uncontrolled growth and very high mutation rate) the study has been difficult [2]. With changing the activation of certain genes, the idea is that the cancer cell will be unable to outcompete healthy cells and thus destroy the cancer.

Although prior research has already been conducted on gene activations that promote or inhibit cancerous growth, many of them have failed to draw a conclusive answer as to the actions of many mechanisms through which the cancerous cell propagates. Many current methods are still actively being explored, such as the effects of Profilin-1 (Pfn-1) on growth but its method of action is as of yet not fully understood. Preliminary research shows that Pfn-1’s role in cancer is significant and a promising target for study in cancer motility [3].

It is hypothesized that Pfn-1’s effects on certain signaling pathways: SMAD3, ERK, and FAK contribute to its method of action. Based off the previous literature on the effects of Pfn-1, more information would be required on its method of action. Additionally, it is known that there are differences in gene expression between 2D and 3D cell cultures. A 3D culture would be more representative of the conditions of a human body and could help us glean more insight into the nature of some of these actions. It is hypothesized that knocking down Pfn-1 would in some way alter the activation of these genes and thus impact the overall action, and that there may be observable differences between 2D and 3D cultures. Knowledge on these effects would greatly help advance cancer research and ultimately boost future treatment options and help cancer patients.

OBJECTIVE
To accomplish this objective, a group of cells with the Pfn-1 gene knocked down were compared with a control group for differences in expression of the following genes in 2D and 3D culture: SMAD3, ERK, and FAK.

HYPOTHESIS/SUCCESS CRITERIA
It is hypothesized that the knockdown of Pfn1 will alter the activation of the pathways SMAD3, ERK and FAK. The amount of the knockdown of Pfn1 is first measured by a Western Blot. After confirming the knockdown is efficient, then the other genes would have their respective protein levels measured also through a Western Blot. Should the amount of these proteins change significantly between the control (L2 cells) and knockdown (P1 cells), then it can be said that the success criteria have been met and that changing the Pfn1 levels has effected the levels of the other proteins.

METHOD
The study was designed to look at the changes in relative expression of SMAD3, ERK, and FAK between the L2 control group of cells and the P1 Pfn1 knocked down cells as well as differences between 2D and 3D cultures. The cells used were MDA-MB-231 triple negative breast cancer, with the only difference between the groups being a knockdown of Pfn-1 via an shRNA into the P1 group of cells. In the 2D case, cells were seeded onto a tissue culture dish with area 60mm^2 and allowed to grow for 3 days’ time. The 3D case was done similarly, with the only difference being the addition of Matrigel onto the tissue culture dish prior to seeding the cells. Once both groups had finished growing for the set period of time, the cells were harvested, lysed in a manner that left the proteins intact, and finally boiled. Once the boiling was complete and the cell samples cooled down, they were loaded into a Western Blotting apparatus with a 12% gel. A gel's percentage is a measure of its concentration of Acrylamide, which provides resistance to the movement of proteins through the gel; a higher percentage gel provides more resistance to the protein movement, especially those of a higher weight. This specific gel percentage is ideal to see the separation between these specific proteins due to their weight differences. Once the blot run, the results of the gel were transferred to a membrane which then had a solution of antibodies added to be able to visualize the protein levels. The antibodies bound to the proteins and would fluoresce in the presence of an ECL solution under the BioRad Imager. The imager would then capture this photographic information, and ImageJ was used to determine the relative intensity of the bands quantitatively. These numerical values were then imported into Microsoft Excel, where it was then normalized and plotted to allow easier and more meaningful interpretation of the data.

The variables to be analyzed from this data are the relative intensity of the bands. The activated forms of the proteins are the phosphorylated forms (denoted with a p- prefix onto the protein name). It is important to pay attention to these as well as the total overall amount of protein (not denoted with anything other than the protein name). The Excel data gives us these numerical values we seek and allows easy interpretation of the results of the experiment. It was important to see the relative amount of SMAD3, ERK, and FAK between the L2 and P1 cells; this comparison was done in 2D and 3D to ascertain a more conclusive answer.
RESULTS

Figure 1. The amount of Pfn1 knockdown between two sets of cells (Set 1, S1 and Set 2, S2). Only one trial was done, no error bars or \( n \) value shown.

Figure 2. The amounts of ERK versus activated ERK (pERK) in a Pfn1 knockdown condition. Only one trial was done, no error bars or \( n \) value shown.

Figure 3. The amounts of FAK versus activated FAK (pFAK) in a Pfn1 knockdown condition. Only one trial was done, no error bars or \( n \) value shown.

All values were as reported by ImageJ under default configuration. The data were imported into Excel and then plotted as shown above. Each set of cell groups is denoted as either S1 for Set 1 or S2 for Set 2. Each set was harvested on different days. In each case, only one trial was done and is shown, thus no error bars or \( n \) value denoting the number of trials is shown. Clean SMAD3 data was not obtainable and is thus not shown here.

DISCUSSION

The results of this study has not yet provided conclusive evidence as to the correlation between Pfn1 and SMAD3, ERK, or FAK. The quality of the P1 cell's knockdown was efficient and measurably significant. General trends for ERK and FAK in their inactive form seem to be unchanged. This is the expected behavior due to the fact that only the activated form should change when the Pfn1 gene is knocked down. From this, it can be said that the total protein amount is accurate. Overall, not enough data was collected to conclusively state the relationship between the activated forms of the genes.

The activated ERK groups (pERK) seemed to increase through the knockdown. The activated FAK also seems to show this trend of increased activity with the knockdown of Pfn1. SMAD3 was rather messy and could not be captured without excessive amounts of background noise, and we are still working to fix this issue. It should be an area of future study and continued research to properly identify its change in response to a Pfn1 knockdown.

This study was limited by several factors. Firstly, the data set was rather small, with few trials performed for each protein. The antibodies used could have been old, and this would cause a lesser amount of binding than should occur with fresh antibodies. Furthermore, antibodies from different companies could also produce different binding results. Some combination of these various bindings could affect the amount of protein that fluoresces as only those with bound antibodies can be revealed by the imager. Such an issue could also plague the SMAD3 results, which was very messy due to the potential nonspecificity of the used antibody.

In the future, additional trials could be run for each protein. Additionally, the antibodies could be replaced with fresh ones for the sake of this study.

CONCLUSION

Cancer is a very serious medical and economic problem. Insight into the gene activation and mechanisms may allow us to better fight this disease in the future. From this study, we can design new ones to focus more closely on the relationship between Pfn1 and the genes in question to see its effects through the gathering of larger amounts of data.

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REFERENCES
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